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A Test of Archonta Monophyly and the Phylogenetic Utility of the Mitochondrial Gene 12S rRNA

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ABSTRACT The relationships within the superorder Archonta, which contains the orders Dermoptera (flying lemurs), Scandentia (tree shrews), Chiroptera (bats), and Primates, were examined through the analysis of five newly derived and complete mitochondrial 12S rRNA sequences. The new data is combined with 83 additional known mammalian sequences to provide a full phylogenetic sampling. Phylogenetic hypotheses are generated using PAUP 3.1.1 (Swofford [1993] Illinois Natural History Survey, Champaign, IL) through analyses of all characters equally weighted, transversions only, and the effect of alignment gaps on phylogeny. The Parsimony Jackknifer (Farris et al. [1996] Cladistics *12*:99–124) was used to assess the level of ambiguity present in the sequence data, and therefore the strength of the tree topologies. The conclusions of Springer and Douzery (1996, J. Mol. Evol. *43*:357–373) which states that 12S rRNA is reliable to a time depth of 100 mya is unsupported by these analyses. The usefulness of 12S rRNA to aid in solving Archonta relationships and others of similar time depth is found to be suspect. Am J Phys Anthropol 107:225–241, 1998.

The determination of a sister taxon to the order Primates has proven to be a very controversial and complicated task. The relationships within the superorder Archonta, which contains the orders Dermoptera (flying lemurs), Scandentia (tree shrews), Chiroptera (bats), and Primates, have been held up to intense scrutiny. Such scrutiny has led almost all molecular researchers to conclude that Archonta is polyphyletic with a monophyletic Chiroptera more basal on the eutherian tree. Relationships of the relevant taxa to each other, and within the context of other closely related eutherian mammals, are without consensus (Allard et al., 1996).

DETERMINATION OF ARCHONTAN RELATIONSHIPS

Much of the flux of research into Archontan relationships stems from a reaction to the phylogenetic hypothesis of Pettigrew (1986, 1991a, 1991b) and Pettigrew et al. (1989) who contend that the only characters grouping the two bat suborders are ones functionally associated with flight. There has been little support for this assertion, as all subsequent tests of bat monophyly, both morphological (Beard, 1993; Johnson and Kirsch, 1993; Luckett, 1993; Novacek, 1990, 1992a,b, 1994; Simmons, 1993; Wible and Novacek, 1988) and molecular (Adkins and Honeycutt, 1991, 1993; Ammerman and Hillis, 1992; Bailey et al., 1992; Bennett et al., 1988; Honeycutt and Adkins, 1993; Mindell et al., 1991; Novacek, 1994; Springer

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and Kirsch, 1993; Stanhope et al., 1992: Thewissen and Babcock, 1993; Wible and Martin, 1993), have supported chiropteran monophyly.

Beard (1990, 1993) (Fig. 1B) and Kay et al. (1990) suggest that Primates are the sister taxon to Dermoptera. Novacek (1992b, 1994) published a more comprehensive analysis with morphological characters and when he analyzes the evidence without the fossil specimens, the sister taxon to Primates shifts to Scandentia. With morphological characters alone, and tandemly aligned with Adkins and Honeycutt's (1991) data from the mitochondrial gene cytochrome *c* oxidase subunit II (COII), Dermoptera is found to be sister to Chiroptera (supporting the superorder Volantia), and Primates are sister to Scandentia (Novacek 1994; Fig. 1C). This combined molecular and morphological data also support a monophyletic Archonta.

Molecular data from a mitochondrial 12S rRNA fragment (Ammerman and Hillis, 1992), and exon 28 of the von Willebrand factor gene (Porter et al., 1996) find the sister to Primates to be Dermoptera, in support of Beard (Fig. 1D,E). Porter et al. (1996) also support a sister relationship between Scandentia and Lagomorpha, and bats are monophyletic. COII sequences (Adkins and Honeycutt, 1993) find Primates to be sister to a ((Scandentia, Macroscelidea)Dermoptera) clade (Fig. 1F). The COII and von Willebrand factor genes test the monophyly of Archonta and find it to be polyphyletic.

Two other nuclear genes and proteins have been used to test these relationships as well. These include epsilon-globin (Bailey et al., 1992), and a fragment from exon 1 of the gene coding for interphotoreceptor retinoid-binding protein (IRBP) (Stanhope et al., 1992). The epsilon-globin results of Bailey et al. (1992) (Fig. 1G) show a sister relationship between Lagomorpha and Scandentia, which is in turn sister to Primates, and the entire aforementioned clade is sister to Dermoptera. The IRBP findings of Stanhope et al. (1992) also show a polyphyletic Archonta, and has, yet again, a different sister clade to Primates (Fig. 1H). This network shows a clade nesting the sisters Lagomorpha and Rodentia to the sisters Dermoptera and Scandentia, and that whole clade is sister to Primates. Honeycutt and Adkins (1993) combined the COII, IRBP, and epsilon-globin sequences into a total evidence alignment and in a transversion parsimony tree found Scandentia sister to Dermoptera with that clade sister to Primates (Fig. 1I).

PURPOSE OF THIS STUDY

We sequenced the complete mitochondrial gene 12S rRNA in five new taxa from the five orders (approximately 1,000 bp each): *Cynocephalus variegatus* (Dermoptera), *Rousettus leschenaulti* (Chiroptera), *Tupaia glis* (Scandentia), *Tarsier bancanus* (Primates), and *Tenrec ecaudatus* (Insectivora). This provides sequences for each member of the proposed superorder Archonta, and adds to an already very large published database. This analysis allowed us to pursue two goals: 1) to attempt to provide further resolution of the problem of determining a sister order to Primates; and 2) in the process, this large 12S rRNA sequence database can be examined for its overall phylogenetic utility in uncovering ordinal relationships, thereby testing the assertions of Springer and Douzery (1996).

MATERIALS AND METHODS

Laboratory methods

Purified DNA samples were kindly provided by Rodney L. Honeycutt of Texas A&M University for *Cynocephalus variegatus* (Dermoptera), *Rousettus leschenaulti* (Chiroptera), and *Tupaia glis* (Scandentia). Dr. Don Nichols of the National Zoological Park of Washington, DC permitted access to liver tissue of *Tarsier bancanus* (specimen number NZPP92–152). *Tenrec ecaudatus* tissue (specimen number 3696) was obtained from the Smithsonian Institution. DNA was extracted from the tissue using the protocol of Longmire et al. (1992). In this method, .30 to .40 grams of tissue was macerated, placed in a lysis buffer, and treated with proteinase K. Rather than using dialysis to clean the extracted DNA, as suggested in the protocol, we performed a phenol/chloroform extraction followed by chloroform extraction. Primers for use in PCR amplification (Table 1 and

Fig. 2) were developed by identifying mammalian conserved regions in tRNA Val, 12S rRNA, tRNA Phe, and 16S rRNA between an alignment of 14 taxa. PCR templates used in sequencing were obtained by using Perkin-Elmer (Norwalk, CT) kits and the primers were annealed at temperatures between 55–60°C. Each 50 µl PCR product was cleaned with 4 mls 0.1 × TE according to Centricon protocols. Sequencing was performed in both directions on the templates using Life Technologies (Gaithersburg, MD) protocol and dsDNA Cycle Sequencing kits. Radioactive labeling of the primers was done with gamma P-32. The sequencing products were run on 1 × TBE 4% acrylamide gels at a high temperature of approximately 55–60°C. Gels were dried at 80°C for one-half to an hour and placed in cassettes with Kodak BioMax film for approximately two days. Each sequence was read, proofed, and aligned using MacVector sequence analysis software and AssemblyLIGN sequence assembly software by Eastman Kodak Company. Alignment of the various fragments into the five complete sequences was performed using AssemblyLIGN sequence assembly software also by Eastman Kodak Company.

The 12S rRNA sequence data for the above five taxa were combined with 83 other known mammalian sequences (Table 2) and aligned "by eye" for most of its length. Conserved regions were identified as anchors and gaps inserted in regions of variability to maximize matches to the sequences which were longer, and thus forcing the insertion of gap events in a given region. After maximizing a match for each taxon, a final edit was performed to align gaps of equal length. This was done to minimize the number of hypothesized gap events. The taxon, for a given gap length, which had the maximum match to the taxon forcing the gap, was used to determine the placement of other gaps of equal length. In the alignment, this can be seen as gaps of equal length that are aligned under one another. A small region from bases 942–1007 was aligned using the multiple sequence alignment program Clustal W version 1.6 (Thompson et al., 1994). This segment had too high a variability to be aligned by eye. The full alignment with gap-coding (reviewed below) is available from the EMBL server by e-mailing the request (GET ALIGN: DS35643. DAT) to NetServ@EBI.AC.UK.

Data analysis

The sequence data were analyzed in several ways using the software Phylogenetic Analysis Using Parsimony (PAUP 3.1.1; Swofford, 1993). Due to the size of the data matrix, and hence the number of possible tree topologies, exhaustive searches could not be performed. However, heuristic methods were employed that look at a very large number of tree topologies. The trees of shortest length are kept and tree statistics such as the rescaled consistency index (RC) (Farris, 1989) were used where possible. Also employed was Bremer's support (BS; Bremer, 1988) method of evaluating the strength of phylogenetic hypotheses by looking at how many additional steps were necessary to break up the relationships found in the shortest tree(s). Computing and saving these additional trees for this dataset is very time-intensive and, consequently, the possibility that BSs lower than those found are likely to exist. The Parsimony Jackknifer 4.22 (Farris et al., 1995) was used to look at the amount of ambiguity present in the data matrix in order to determine if the tree topologies obtained from the heuristic searches employed with PAUP were well supported. In all analyses the gaps were treated as missing data. The marsupials *D. virginiana*, and the *M. giganteus* were used as outgroups.

To simplify the discussion of tree topologies and the accompanying figures, we will refer only to the highest taxonomic levels found to be monophyletic in each analysis. In the figures, members of Archonta are presented in bold for easier identification. Individual taxon used to represent the various orders can be identified by referring to Table 2.

RESULTS AND DISCUSSION

Equally weighted data analysis

The data were first analyzed using tree-bisection-reconnection (TBR) branch-swapping and a random stepwise addition of taxa for 100 replicates with all characters equally

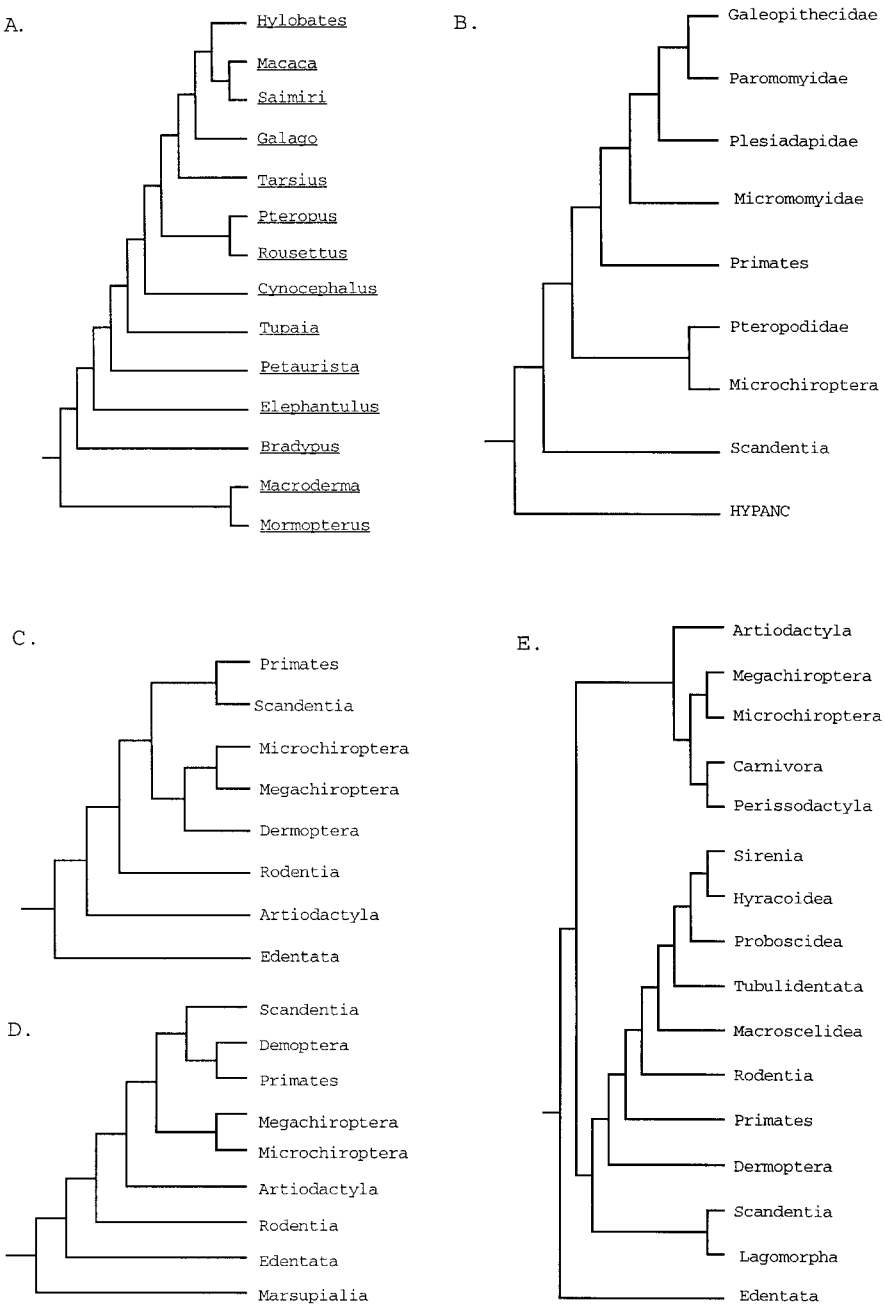

Fig. 1.

weighted. Three equally parsimonious trees with lengths of 6,536 steps and an RC of 0.104 were found and a strict consensus tree computed (Fig. 3). Archonta is found to be polyphyletic with Dermoptera falling within the order Primates (node 1). The family Tarsiidae is shown to be more basal than Dermoptera. The clade sister to the Primates/Dermoptera grouping contains the rodent family Gliridae in a sister relationship

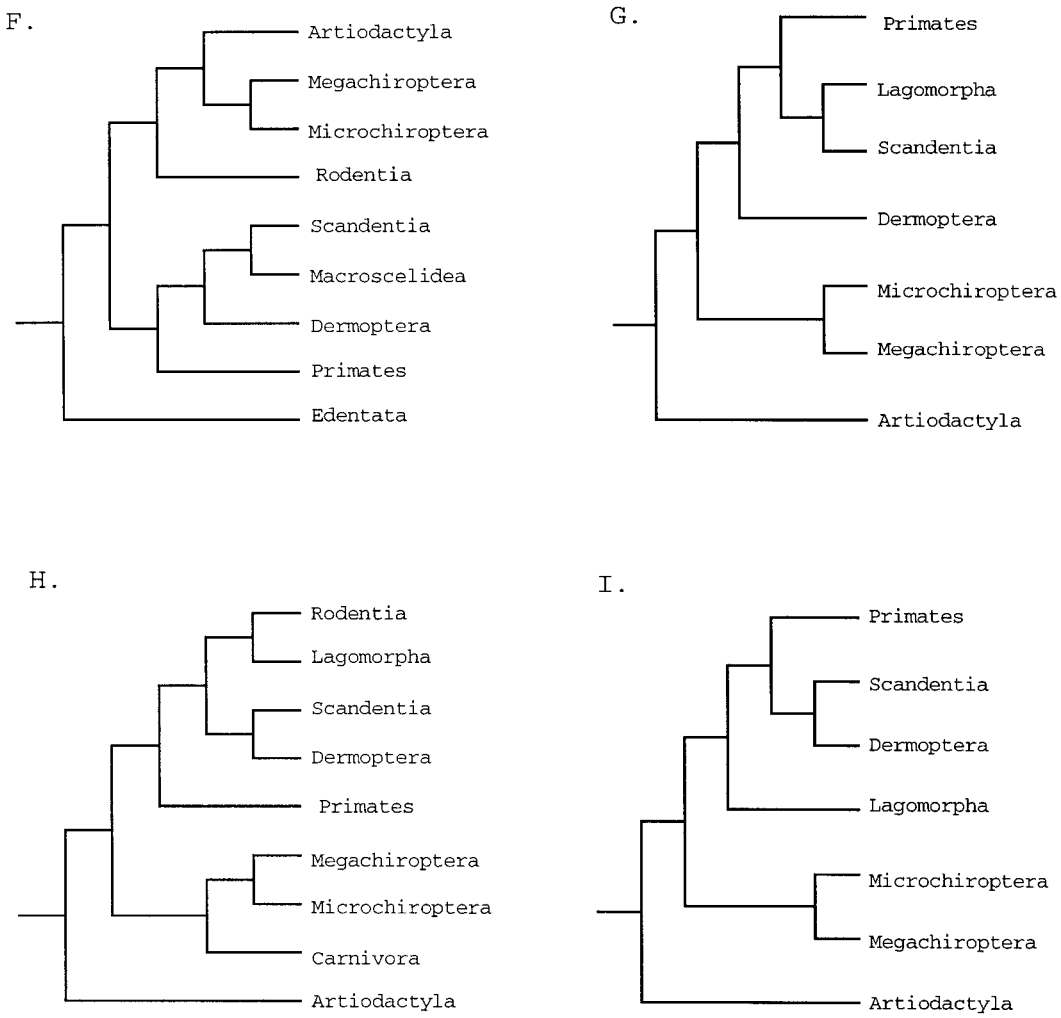

Fig. 1. Summary of published phylogenetic hypotheses. (**A**) Most parsimonious tree from 24 neural characters by Pettigrew (1991a). Tree length is 43 with a CI of 0.907. The taxa are as follows: the first five genera (reading down) are members of Primates: *Hylobates, Macaca, Saimiri, Galago;* followed by two megabats, *Pteropus* and *Rousettus,* belonging to Chiroptera; *Cynocephalus* for Dermoptera; *Tupaia* for Scandentia; *Petaurista* for Rodentia; *Elephantulus* for Macroscelidea; *Bradypus* for Edentata; and two microbats, *Macroderma* and *Mormopterus,* also belonging to Chiroptera. (**B**) Most parsimonious tree from 29 morphological characters by Beard (1993). Tree length is 49 with a CI of 0.878. (**C**) (**C–I** are adapted from Allard et al. (1996)). Most parsimonious tree from 49 morphological characters (Novacek, 1994) tandemly aligned to 258 informative transversions from the mitochondrial gene cytochrome *c* oxidase subunit II (COII). (**D**) Most parsimonious tree from a 257-bp fragment of the mitochondrial gene 12S rRNA by Ammerman and Hillis (1992). Tree length equals 293. (**E**) Strict consensus tree from exon 28 of the von Willebrand factor gene (Porter et al., 1996). (**F**) Most parsimonious tree from transversions for COII (Adkins and Honeycutt, 1993). Tree length is 553. (**G**) Most parsimonious tree from the nuclear gene epsilon-globin by Bailey et al. (1992). Tree length is 3,614 steps. (**H**) Consensus tree from exon 1 of the nuclear gene IRBP (Stanhope et al., 1992). (**I**) Most parsimonious tree from tandemly aligned COII, IRBP, and epsilon-globin by Honeycutt and Adkins (1993). Tree length is 1,839.

to the Chiroptera suborder Microchiroptera (node 2). This placement of the microbat breaks up the monophyly of Chiroptera with Megachiroptera (branch 3) in a sister relationship to a large clade containing a polyphyletic Artiodactyla, and a monophyletic Cetacea, Perissodactyla, Carnivora, and Pinnipedia (node 4). Scandentia has a sister

TABLE 1. Polymerase chain reaction (PCR) and sequencing primers are listed 5′ to 3′ and are presented schematically in Figure 2

Name	Sequence
1S	CAA AGC AAG GCA CTG AAA ATG
2′	ATC GTA TGA CCG CGG TGG CTG GCA
2	TCG TGC CAG CCA CCG CGG TCA TAC GAT
2′NS	AAG CAC CGC CAA GTC CTT TGA GTT
2NS	AAA ACT CAA AGG ACT TGG CGG TGC
2GW	TGG GAA GAA ATG GGC TAC ATT
2′U	TTA GTT TAC TAC TAA ATC CTC CTT
3′GW	TCT TTC ATC TTT CCC TTG CGG TAC T
3′OP	TGA AAT CTT CTA GGT GTA

relationship to Lagomorpha (node 5), and they in turn are sister to the insectivore family Erinaceidae (node 6). This clade is sister to a group containing a monophyletic Sirenia, Hyracoidea, Proboscidea, and members of Insectivora and Rodentia (node 7). Throughout the tree where monophyly was testable, it failed to be supported in the artiodactyls, chiropterans, insectivores, primates, and rodents.

Another replicate of TBR branch-swapping with stepwise random addition of taxa was performed and all trees from one to ten steps longer than the shortest trees of 6536 were kept. Within one step the megabats have a new diverse sister clade (BS = 1). At three additional steps, the sister relationship between Scandentia and Lagomorpha dissolves, and the Microchiroptera and Gliridae rodent clade is no longer sister to the Primates and Dermoptera grouping (BS = 3). The family Tarsiidae leaves the other primates and Dermoptera with the addition of six steps (BS = 6). One relationship of interest that did not collapse within the ten extra steps examined is that of Dermoptera to the primate families Hominidae and Hylobatidae.

Weighted data: transversions, gap-coding, and successive approximations

Two types of transversion analyses were performed on the data. By only looking at transversions (transitions are given a weight of 0), we are using a model of evolutionary change in which it is held that transversions, a change from a purine to a pyrimidine or vice versa, is less likely to occur than transitions, a mutation from purine to purine or pyrimidine to pyrimidine (Higuchi et al., 1984). It is suggested that with a long time span, there would be multiple transitional mutations in single sites, a phenomenon known as saturation. The eutherian radiation considered here, at approximately 60 mya, is a candidate for the down-weighting of transitions. The first type of transversion analysis used a simple equate macro (A = G and C = T), in which the program treats any change from a purine to a pyrimidine or vice versa as a single character change. Early in the tree search, the number of equally parsimonious trees became so high (<28,100) that computer memory could no longer track possible new tree lengths. This lack of resolution most likely resulted from the loss of specific character information.

A second way to look at transversions is to use a step matrix. As in the first transversion analysis, transitions are weighted zero, but types of transversions are kept distinct when mapping characters onto the tree. A heuristic replicate with (TBR) branch-swapping and a stepwise random addition of taxa was performed, which resulted in 203 equally parsimonious trees. These trees were saved and more TBR branch-swapping operations performed on them in which 174 more trees of the same length were found. A strict consensus tree was computed from the 377 trees, each of which had a length of 2,669. Archonta is again polyphyletic (Fig. 4), and the monophyly of the order Primates is broken up by a clade containing the insectivore family Erinaceidae and the suborder Microchiroptera (node 1). Megachiroptera is shown in a sister relationship to Pholidota (node 2), and that clade in turn is part of a polytomy (node 3) which includes, among many others, the primate-containing clade. Scandentia is sister to the insectivore family Tenrecidae (node 4), and Dermoptera is sister to a clade containing the rodent families Gliridae, Caviidae, and Hydrochaeridae (node 5). The sister clade to the one containing Dermoptera and the rodents includes an insectivore from the family Chrysochloridae and a monophyletic Sirenia, Proboscidea, and Hyracoidea (node 6). Where monophyly could be tested, the results were the same as with all characters equally weighted.

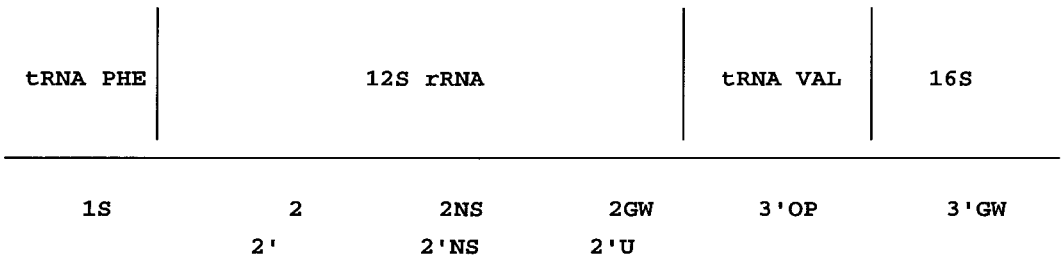

Fig. 2. Schematic of primer placement within the mitochondria.

Another replicate of TBR branch-swapping with stepwise random addition of taxa was performed and all trees from one to three steps longer than the shortest trees of 2,669 were kept. Performing the tree search for those within ten steps was not possible due to enormous computational requirements. With two additional steps the Tenrecidae insectivore and Scandentia clade changes sister group (BS = 2), and at three steps the Pholidota and Megachiroptera bat clade moves (BS = 3). As noted before, it is highly likely that other trees exist to lower these BS numbers further.

Finally, to investigate if the phylogenetic information available in gaps could improve resolution, all but two gap regions were coded as individual character states for each taxon (Table 3 identifies all gap regions and codes) and added to the alignment matrix. The character state equals the length of a given gap from 0 to 12. Gap length is used to define the character in order to minimize hypotheses of insertion and deletion events. The analysis with all characters equally weighted was performed a second time with this added coding. The gap regions were retained as missing data in order to preserve any phylogenetic information present in the DNA bases. The same type of heuristic analysis was performed for 62 replicates and a single tree was found with 6,870 steps and an RC of 0.105. The addition of these 43 characters yielded changes in tree topology from the first analysis (Fig. 5). Archonta remains polyphyletic and Dermoptera still sits within the Primates (node 1). This group is sister to a clade containing the bat suborder Microchiroptera in a sister relationship to the rodent family Gliridae, which are in turn sister to the rodent family Muridae (node 2). Scandentia also remains sister to Lagomorpha (node 3), but their grouping is now more basal on the tree. The bat suborder Megachiroptera is sister to a clade containing many artiodactyls, as well as Edentata and Pholidota (node 4).

Another replicate of TBR branch-swapping with stepwise random addition of taxa was performed and all trees from one to ten steps longer than the shortest trees of 6,870 were kept. Within four steps the bat suborder Megachiroptera changes sister group, Scandentia and Lagomorpha are no longer sister, and the primate family Tarsiidae leaves the remaining primates and Dermoptera (BS = 4). In ten steps the bat suborder Microchiroptera leaves its sister, the rodent family Gliridae (BS = 10), but as with all characters equally weighted without gap coding, Dermoptera remains with the primate families Hominidae and Hylobatidae.

All three tree topologies find a polyphyletic Archonta and Chiroptera, but vary on the relationship of Primates and Scandentia to the other orders. For all characters with and without gap coding, Primate monophyly is broken up by Dermoptera, and Scandentia is sister to Lagomorpha. For the transversion analysis, the Primates are broken up by the insectivore family Erinaceidae and the bat suborder Microchiroptera, while Scandentia is sister to the insectivore family Tenrecidae. Dermoptera is in a clade with the rodent families Gliridae, Caviidae, and Hydrochaeridae.

Springer and Douzery (1996) analyzed the secondary structure of the 12S gene and

TABLE 2. List of taxa and sources

Order	Family	Species/abbreviation	Genbank accession	Number Reference
Artiodactyla	Antilocapridae	*Antilocapra americana*	M55540	Kraus and Miyamoto (1991)
Artiodactyla	Bovidae	*Aepyceros melampus*	M86496	Allard et al. (1992)
Artiodactyla	Bovidae	*Bos taurus*	J01394	Anderson et al. (1982)
Artiodactyla	Bovidae	*Bos grunniens*	no number	Miyamoto et al. (1989)
Artiodactyla	Bovidae	*Boselaphus tragocamelus*	M86494	Allard et al. (1992)
Artiodactyla	Bovidae	*Bubalus bubalis*	no number	Tanhauser (1985)
Artiodactyla	Bovidae	*Capra hircus*	M55541	Kraus and Miyamoto (1991)
Artiodactyla	Bovidae	*Cephalophus maxwelli*	M86498	Allard et al. (1992)
Artiodactyla	Bovidae	*Damaliscus dorcas*	M86499	Allard et al. (1992)
Artiodactyla	Bovidae	*Gazella thomsoni*	M86501	Allard et al. (1992)
Artiodactyla	Bovidae	*Kobus ellipsiprymnus*	M86497	Allard et al. (1992)
Artiodactyla	Bovidae	*Madoqua kirki*	M86495	Allard et al. (1992)
Artiodactyla	Bovidae	*Oryx gazella*	M86500	Allard et al. (1992)
Artiodactyla	Bovidae	*Tragelaphus imberbis*	M86493	Allard et al. (1992)
Artiodactyla	Cervidae	*Cervus unicolor*	M35875	Miyamoto et al. (1990)
Artiodactyla	Cervidae	*Muntiacus reevesi*	M35877	Miyamoto et al. (1990)
Artiodactyla	Cervidae	*Odocoileus virginianus*	M35874	Miyamoto et al. (1990)
Artiodactyla	Cervidae	*Hydropotes inermus*	M35876	Miyamoto et al. (1990)
Artiodactyla	Giraffidae	*Giraffa camelopardalis*	no number	Tanhauser (1985)
Artiodactyla	Suidae	*Sus scrofa*	no number	Tanhauser (1985)
Artiodactyla	Tayassuidae	*Tayassu tajacu*	X86944	Douzery and Catzeflis (1995)
Artiodactyla	Tragulidae	*Tragulua napu*	M55539	Kraus and Miyamoto (1991)
Carnivora	Felidae	*Felis concolor*	U33495	Springer et al. (1995)
Carnivora	Felidae	*Felis catus*	U20753	Lopez et al. (1996)
Cetacea	Balaenopteridae	*Balaenoptera physalus*	X61145	Arnason et al. (1991)
Cetacea	Balaenopteridae	*Balaenoptera musculus*	X72204	Arnason and Gullberg (1993)
Cetacea	Delphinidae	*Stenella coeruleoalba*	X78168	Douzery (1993)
Cetacea	Physteridae	*Physeter macrocephalus*	no number	Arnason et al. (1991)
Chiroptera				
Megachiroptera	Pteropodidae	*Rousettus leschenaulti*	This article	
Megachiroptera	Pteropodidae	*Nyctimene albiventer*	U61077	Springer and Douzery (1996)
Microchiroptera	Vespertilionidae	*Eptescius fuscus*	U61082	Springer and Douzery (1996)
Dermoptera	Cynocephalidae	*Cynocephalus variegatus*	This article	
Edentata	Dasypodidae	*Chaetophractus villosus*	U61080	Springer and Douzery (1996)
Hyracoidea	Procaviidae	*Procavia capensis*	U60184	Lavergne et al. (1996)
Hyracoidea	Procaviidae	*Dendrohyrax dorsalis*	X86941	Douzery and Catzeflis (1995)
Insectivora	Chrysochloridae	*Amblysomus hottentotus*	M95108	Allard and Miyamoto (1992)
Insectivora	Erinaceidae	*Atelerix albiventris*	M95109	Allard and Miyamoto (1992)
Insectivora	Erinaceidae	*Erinaceus europaeus*	X88898	Krettek et al. (1995)
Insectivora	Tenrecidae	*Tenrec ecaudatus*	This article	
Insectivora	Soricidae	*Blarina brevicauda*	M95110	Allard and Miyamoto (1992)
Lagomorpha	Leporidae	*Oryctolagus cuniculus*	Web Site www.ba.cnr.it/guineapig.html	
Marsupialia	Didelphidae	*Didelphis virginiana*	Z29573	Janke et al. (1994)
Marsupialia	Macropodidae	*Macropus giganteus*	X86941	Douzery and Catzeflis (1995)
Marsupialia	Microbiotheriidae	*Dromiciops gliroides*	U61073	Springer and Douzery (1996)
Perissodactyla	Equidae	*Equus caballus*	X79547	Xu and Arnason (1994)
Perissodactyla	Equidae	*Equus grevyi*	X86943	Douzery and Catzeflis (1995)
Perissodactyla	Rhinocerotidae	*Ceratotherium simum*	X86942	Douzery and Catzeflis (1995)
Perissodactyla	Rhinocerotidae	*Rhinoceros unicornis*	X97336	Xu et al. (1996)
Pholidota	Manidae	*Manis* sp.	U61079	Springer and Douzery (1996)
Pinnipedia	Phocidae	*Halichoerus grypus*	X72004	Arnason et al. (1993)
Pinnipedia	Phocidae	*Phoca vitulina*	X63726	Arnason and Johnson (1992)
Primates	Hominidae	*Gorilla gorilla*	X93347	Xu and Arnason (1996)
Primates	Hominidae	*Homo sapiens*	J01415	Anderson et al. (1981)
Primates	Hominidae	*Pan paniscus*	D38116	Hixson and Brown (1986)
Primates	Hominidae	*Pan troglodytes*	X93335	Arnason et al. (1996)
Primates	Hominidae	*Pongo pygmaeus*	no number	Hixson and Brown (1986)
Primates	Hylobatidae	*Hylobates lar*	X99256	Arnason et al. (1996)
Primates	Tarsiidae	*Tarsius bancanus*	This article	
Proboscidea	Elephantidae	*Elephas maximus*	no number	Lavergne et al. (1996)
Proboscidea	Elephantidae	*Loxodonta africana*	U60182	Lavergne et al. (1996)
Rodentia	Caviidae	*Cavia porcellus*	L35585	Frye and Hedges (1995)
Rodentia	Gliridae	*Glis glis*	X84385	Hanni et al. (unpub.)
Rodentia	Gliridae	*Muscardinus avellanarius*	X84384	Hanni et al. (unpub.)
Rodentia	Hydrochaeridae	*Hydrochaeris hydrochaeris*	U61081	Springer and Douzery (1996)
Rodentia	Muridae	*Acomys cahirinus*	X84387	Hanni et al. (unpub.)
Rodentia	Muridae	*Cricetomys gambianus*	X99461	Dubois et al. (unpub.)
Rodentia	Muridae	*Cricetulus migratorius*	X84389	Hanni et al. (unpub.)

TABLE 2. (continued)

Order	Family	Species/abbreviation	Genbank accession Number	Reference
Rodentia	Muridae	*Hylomyscus stella*	X85953	Sourrouille et al. (unpub.)
Rodentia	Muridae	*Leopoldamys edwarsi*	X84386	Hanni et al. (unpub.)
Rodentia	Muridae	*Mastomys erythroleucus*	X85952	Sourrouille et al. (unpub.)
Rodentia	Muridae	*Mesocricetus auratus*	X84390	Hanni et al. (unpub.)
Rodentia	Muridae	*Microtus nivalus*	X99464	Dubois et al. (unpub.)
Rodentia	Muridae	*Mus cookii*	X85946	Sourouille et al. (unpub.)
Rodentia	Muridae	*Mus crociduroides*	X85951	Sourouille et al. (unpub.)
Rodentia	Muridae	*Mus mattheyi*	X85950	Sourrouille et al. (unpub.)
Rodentia	Muridae	*Mus musculus*	J01420	Bibb et al. (1981)
Rodentia	Muridae	*Mus pahari*	X84383	Hanni et al. (unpub.)
Rodentia	Muridae	*Mus platythrix*	X85947	Sourrouille et al. (unpub.)
Rodentia	Muridae	*Mus saxicola*	X85948	Sourrouille et al. (unpub.)
Rodentia	Muridae	*Mus setulosus*	X85949	Sourouille et al. (unpub.)
Rodentia	Muridae	*Nesomys rufus*	X99462	Dubois et al. (unpub.)
Rodentia	Muridae	*Peromyscus leucopus*	X99463	Dubois et al. (unpub.)
Rodentia	Muridae	*Rattus norvegicus*	X14848	Gadaleta et al. (1989)
Rodentia	Muridae	*Tatera kempi gambiana*	X84391	Hanni et al. (unpub.)
Rodentia	Muridae	*Uranomys ruddi*	X84388	Hanni et al. (unpub.)
Scandentia	Tupaiidae	*Tupaia glis*	This article	
Sirenia	Dugongidae	*Dugong dugon*	U60185	Lavergne et al. (1996)
Sirenia	Trichechidae	*Trichechus manatus*	U60183	Lavergne et al. (1996)

found bias in the patterns of transition versus transversion substitutions in the stems and loops of the molecule. They suggest that throughout the 12S rRNA molecule, saturation occurs for transitions at approximately 20 mya, and that transversions as a whole remain useful to about 100 mya (also see Miyamoto and Boyle, 1989). Their hypothesis suggests that our tree derived from the step matrix will be one derived from characters retaining phylogenetic information, and the two other trees with all characters equally weighted may be ones distorted due to noise caused by saturation. Lack of resolution in the transversion tree for taxa within orders (such as between the great apes) may be a result of the elimination of transitions. Within orders, the divergence times are much smaller and it has been found that within the primates, sequence divergence between the great apes is 87–94% transitions (Hixson and Brown, 1986). Using a model of gene evolution to pick among trees is dangerous, for many reasons. One reason is that models such as Springer and Douzery's (1996) rely on calibration dates from the possibly inaccurate fossil record. By following an incorrect transversion model, valuable transition data may be ignored.

With the measures used so far, the trees obtained with all characters equally weighted with and without gap coding show high levels of homoplasy, as evidenced in the RC for the entire tree (0.105 and 0.104, respectively), and in the BS index for the various nodes (collapse occurring within only 1–10 steps). The problem with using these findings to justify the transversion weighting scheme is that these measures don't test the assertions of Springer and Douzery (1996) that transitions are the main cause of the homoplasy at the time depth of approximately 60 mya. Farris (1969) and Carpenter (1994) promote a character-based method of finding a best tree when several equally parsimonious solutions are found. Characters are reweighted according to how well they fit the original tree topology. In other words, those characters which are highly congruent with others will be given greater weight in a subsequent round of tree building. The resulting tree topology will be one influenced most by those characters showing lower levels of homoplasy. If transitions are responsible for high levels of homoplasy in this dataset of 12S rRNA sequences, then in the reweighting their influence will be minimized. We should then expect that the resulting topology should converge on the transversion analysis. To that end, 19 replicates with TBR branch-swapping and a random stepwise addition of taxa were performed using the reweight characters

command for all characters equally weighted without gap-coding. The RC for each character as derived from the original three most parsimonious trees is used to scale new weights in a range from 0–1,000. One most parsimonious topology resulted (Fig. 6). For the taxa of interest, the successive approximation tree had only two disagreements with the original consensus tree (Fig. 3). With successive approximations, Dermoptera remains sister to the primate families Hominidae and Hylobatidae (node 1, Fig. 6), with that whole clade sister to a Microchiroptera and Gliridae rodent clade (node 2, Figs. 3, 6). The difference is in the placement of the primate family Tarsiidae which, unlike its placement near the Hominidae and Hylobatidae in the consensus tree (node 1, Fig. 3), it is now shown in a sister relationship to an Edentata/Pholidota clade (node 3, Fig. 6). Both trees break up the monophyly of Chiroptera and find Megachiroptera sister to various large multi-clade groupings (node 4, Figs. 3, 6). Both trees also support a sister relationship of Scandentia to Lagomorpha (node 5, Figs. 3, 6), and their sister relationship to the insectivore family Erinaceidae (node 6, Figs. 3, 6). Their respective sister clade (node 7, Figs. 3, 6) is also the same as in the consensus tree. The differences between the consensus and successive weighting trees revolve largely around the placement of the Tarsiidae, with the less homoplasious characters removing the taxon from the rest of the primates.

In contrast to the above results, the transversion consensus tree has several differences from the successive approximation results. In the former (Fig. 4), Scandentia is sister to the insectivore family Tenrecidae; Dermoptera is sister to the rodent families Gliridae, Caviidae, and Hydrochaeridae; Megachiroptera is sister to Pholidota; and Primates is broken up by the Microchirop-

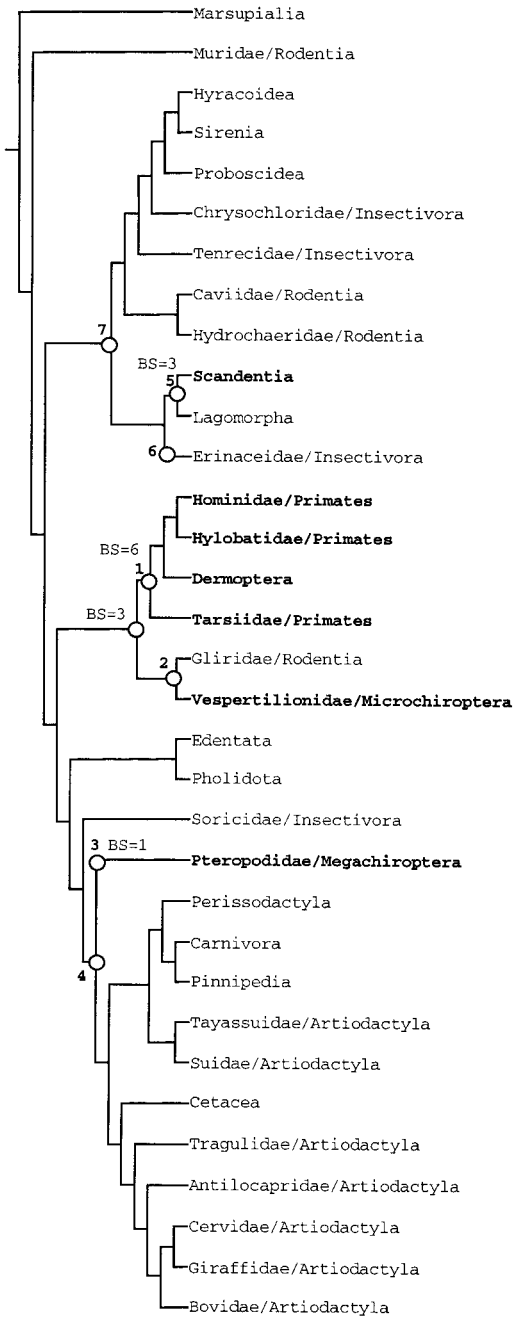

Fig. 3. Strict consensus of three equally parsimonious trees derived from the mitochondrial gene 12S rRNA with all characters equally weighted. A heuristic search with 100 replicates was performed using a random stepwise addition of taxa and branch-swapping. The length of the original three trees is 6,536 steps with an RC of 0.104. Taxa are designated at the highest level of monophyly found in the tree. For example, orders that are monophyletic are given a single branch with the ordinal name; where monophyly is preserved only at the family level, family/order is indicated; and, last, when resolution is found only at the genus level it is indicated as *genus*/family/order. For individual taxon used in the study, see Table 2. Numbers at nodes and BS values refer to text discussion.

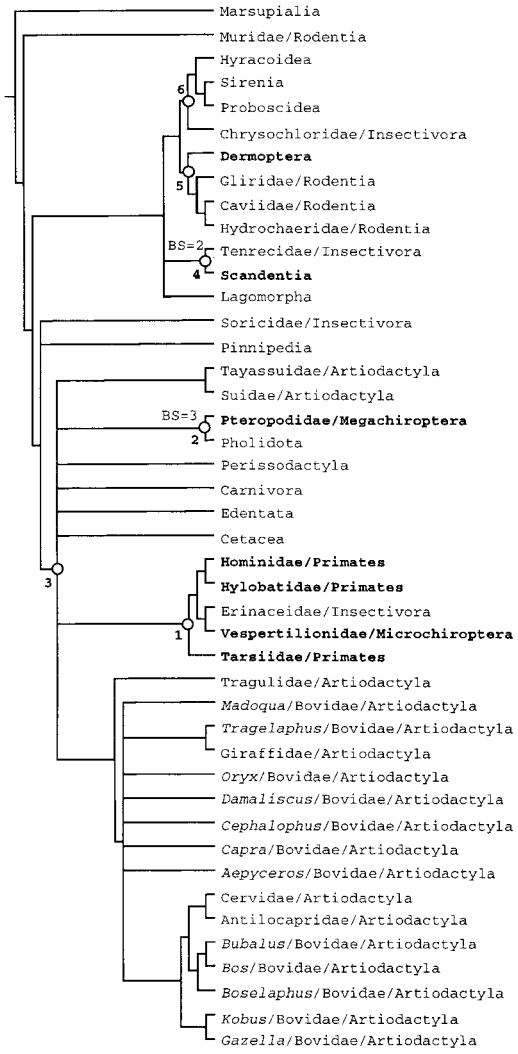

Fig. 4. Strict consensus of 377 equally parsimonious trees derived from a step matrix transversion analysis of the mitochondrial gene 12S rRNA. The length of each of the 377 equally parsimonious trees is 2,669 steps. Taxa and codes are designated as in Figure 3.

TABLE 3. Gap coding

Gap occurence[1]	Gap region[2]	Gap codes used[3]
G1	41	0 1
G2	59–63	0 1 2 3 5
G3	70	0 1
G4	76–77	0 1 2
G5	92–102	0 1 2 3 4 5 6 7 9
G6	110–111	0 2
G7	133–145	0 2 3 4 5 6 7 8 9
G8	167	0 1
G9	178	0 1
G10	183	0 1
G11	237–240	0 2 3 4
G12	247–248	0 3
G13	253	0 1
G14	270	0 1
G15	277	0 1
G16	302–304	0 1 2
G17	330–342	0 2 3 4 5 6 7 8 9 W
G18	386–391	0 1 2 5
G19	402–403	0 1 2
G20	423–428	0 1 2 3 4 5 6
G21	451	0 1
G22	459	0 1
G23	501–509	0 1 2 3 4 6
G24	513–517	0 2 3 4 5
G25	537–538	0 2
G26	548	0 1
G27	563	0 1
G28	580	0 1
G29	640–644	0 4
G30	677–679	0 1 2 3
G31	691	0 1
G32	704–710	0 1 2 3 6
G33	730	0 1
G34	772–779	0 1 2 3
G35	783–808	Not coded[4]
G36	812–813	0 1 2
G37	828–840	0 9 T E W
G38	870	0 1 2
G39	880	0 1
G40	896–897	0 1 2
G41	910	0 1
G42	927	0 1
G43	942–1007	Not coded[5]
G44	1012	0 1
G45	1021	0 1

[1] This refers to the order of gap appearance in the alignment; available by e-mail through the EMBL server at NetServ@EBI.AC.UK with the request (GET ALIGN: DS35643.DAT).
[2] The gap region corresponds to the numbers in the alignment mentioned above.
[3] The numbers used in gap coding refer to the gap length at a given region for a specific taxa from 0 (no gap) to 9 with the following additional codes: T = 10, E = 11, and W = 12. The codes T, E, and W were used for 10, 11, and 12, respectively.
[4] Due to the complexity of this region these characters are treated as missing data.
[5] This region was aligned using Clustal W 1.6 and was treated as missing data.

tera/Erinaceidae insectivore clade. The Tarsiidae is the most basal, but remains in that grouping. This lack of congruence between the transversion and successive approximation tree calls into question the idea that removal of transitions will remove significant levels of homoplasy from the analysis, and that no valuable information will be lost. By using this method of reiterative weighting, it can be seen that there is no justification for eliminating an entire class of data (transitions) based on theories of how evolution occurs. It has been shown in discussions of the use of "total evidence" (Eernisse and Kluge, 1993) that efforts to partition data along these lines can lead to

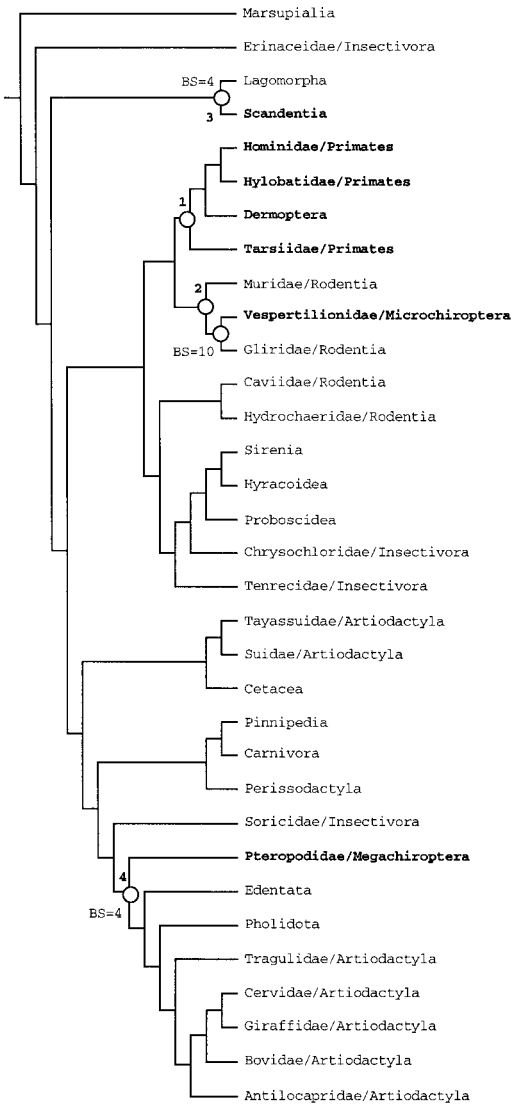

Fig. 5. Most parsimonious tree found with all characters equally weighted and gap-coded for the mitochondrial gene 12S rRNA. A heuristic search with stepwise random addition of taxa and branch-swapping was performed for 62 replicates. The length of the tree is 6,870 steps with an RC of 0.105. Taxa and codes are designated as in Figure 3.

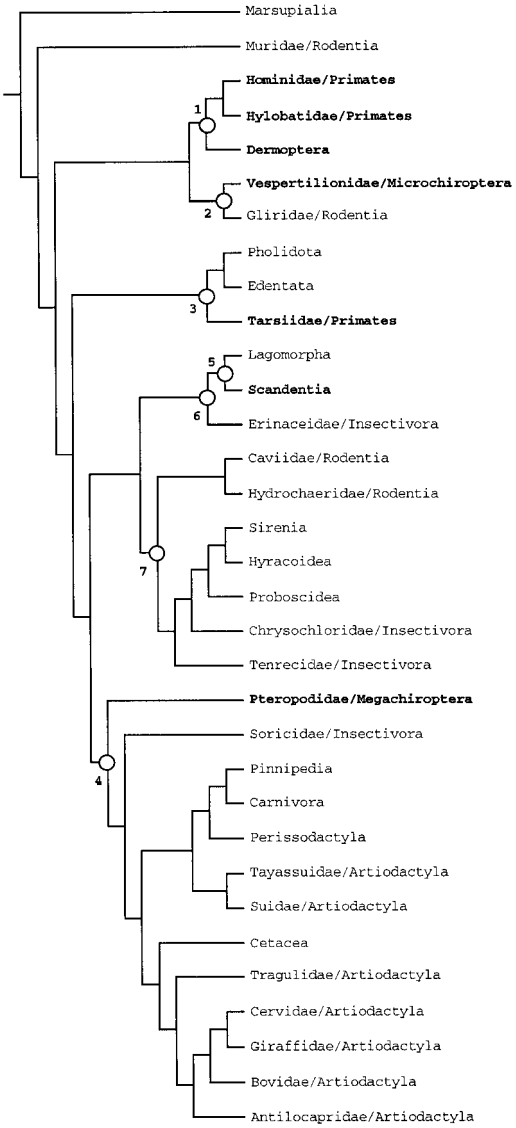

Fig. 6. Successive approximations tree for all characters equally weighted. Characters were reweighted by scaling from 0 to 1,000 for their respective RCs using the trees from which the consensus was computed in Figure 3. Nineteen TBR branch-swapping replicates with random stepwise addition of taxa were performed and one most parsimonious tree found. Taxa and codes are designated as in Figure 3.

erroneous conclusions, such as Eernisse and Kluge (1993) show in their reanalysis of amniote phylogeny. With successive approximation the data speak for themselves, as congruence within the evidence gathered, in this case, the nucleotide bases determines which characters are given added weight in the final analysis.

Data reliability: the parsimony jackknifer

All of the above conclusions are based on tree topologies and, as noted previously, due to the size of this data matrix there is a very high expectation that other shorter and/or

equally parsimonious trees exist that could affect the interpretation presented. Methods such as successive approximation use character congruence to ferret out the more reliable signal. Furthermore, successive approximation does not test the entire matrix for its overall reliability. This problem of basing conclusions on what may be incorrect results due to the time required to calculate topologies, such as with the TBR branch-swapping used above, is addressed by Farris et al. (1996).

To test the matrix for its level of ambiguity, 10,000 replicates were performed with the Parsimony Jackknifer. Figure 7 shows a tree containing groups found in at least 50% of the replicates performed for all characters equally weighted. The results indicate that the 12S rRNA data is highly ambiguous in its ability to solve questions of relationships for the taxa of concern. The jackknifed tree is less resolved overall than the successive weighting tree (Fig. 6), with most of the clades which do hold together coming out of large polytomys. In particular, the jackknifed tree finds the placement of all orders within Archonta ambiguous. This is especially interesting for the placement of Dermoptera, as it had the largest BS (<10) for its placement with the primate families Hominidae and Hylobatidae in Figure 3.

When the same jackknife run is done on a dataset which looks at transversions only, the resolution is just as bad as it is for the all characters jackknife tree (Fig. 8).

These results also contrast with the suggestions of Springer and Douzery (1996) that transversions in 12S rRNA are informative to 100 mya. This gene clearly has high levels of ambiguity throughout its sequence for these deep divergences, and it's utility at this level is suspect. Thus, any comparison of these topologies with the hypotheses shown in Figure 1 is premature. In fact, as noted by Allard and Carpenter (1996) and Eernisse and Kluge (1993), evaluating congruence between tree topologies will not lead to many valuable phylogenetic conclusions, as none of the individual tree topologies yields a most parsimonious solution based on all the combined character data. Instead, now that this large collection of 12S rRNA sequences has been examined in terms of weighting strategies and it's overall phylogenetic utility at this level of divergence, the information can be used to begin further study which can evaluate the most appropriate use of the 12S gene in phylogenetic studies. Previous studies of the 12S rRNA gene have demonstrated that some mammalian deep divergences would be difficult to

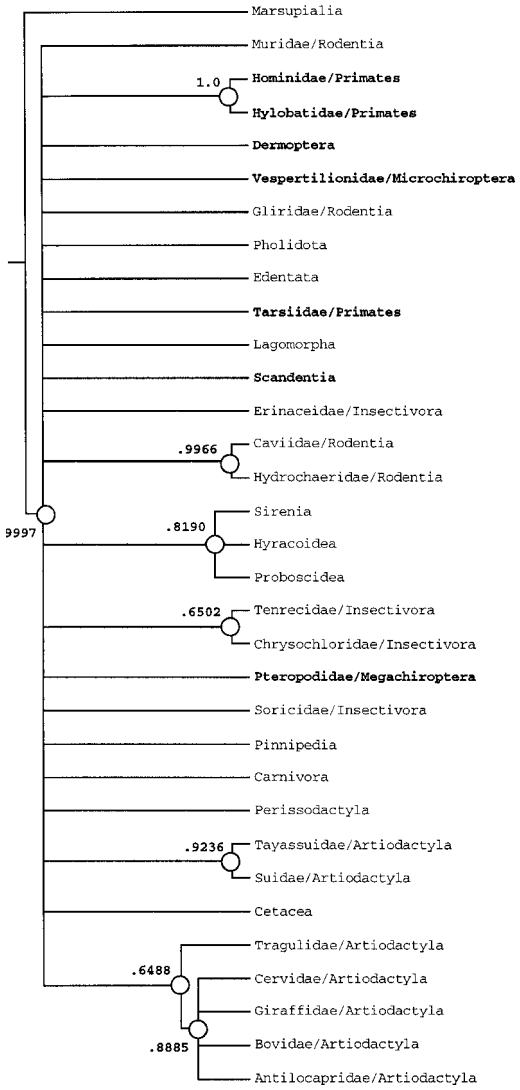

Fig. 7. Parsimony jackknife tree for all characters equally weighted. Numbers at nodes represent the frequency in which the clade appeared in the 10,000 replicates performed. Clades present in less then 50% of the replicates are shown as unresolved, with no specific frequency given. Taxa are designated as in Figure 3.

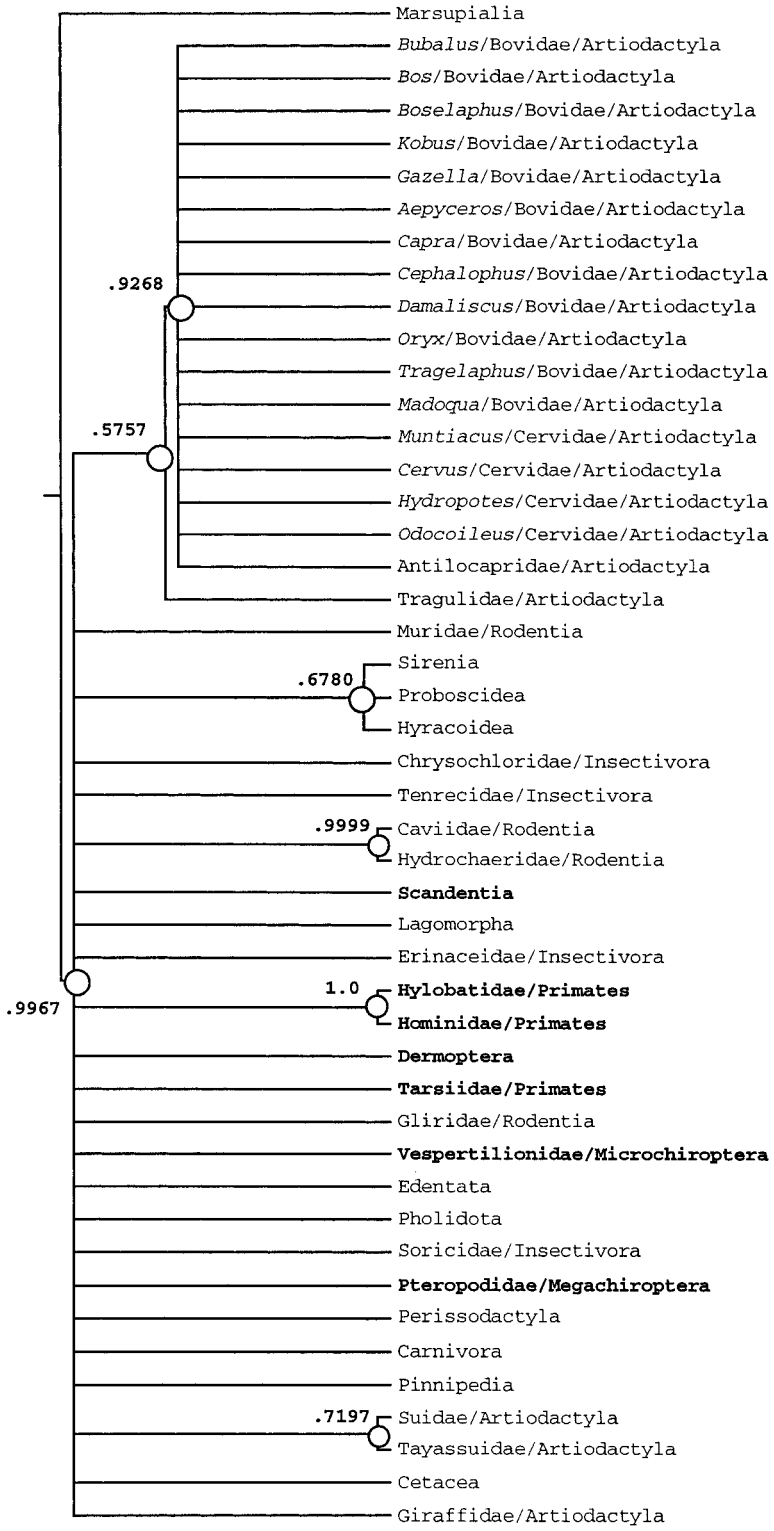

Fig. 8. Parsimony jackknife tree for transversions. Numbers at nodes represent the frequency in which the clade appeared in the 10,000 replicates performed. Clades present in less then 50% of the replicates are shown as unresolved, with no specific frequency given. Taxa are designated as in Figure 3.

capture using this molecule either due to low transition-to-transversion ratios (Nedbal et al., 1994) or due to extreme among-site rate variation (Sullivan et al., 1995).

Examining more taxa and characters is an important goal for mammalian systematists. This study included complete sequences of the 12S rRNA gene and represents one of the largest comparative molecular datasets for this molecule. Earlier predictions concerning the utility of this molecule for uncovering deep mammalian divergences (Springer and Douzery, 1996) were not borne out by the data. Our study demonstrates the need for caution and skepticism of studies which claim great strengths for a particular genetic marker, particularly when the evidence is based on limited sample sizes. Future studies attempting to utilize this molecule for deep mammalian relationships would be wise to combine this gene with other molecular and/or morphological data.

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